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Transfer of vesicles from Schwann cells to axons: a novel mechanism of communication in the peripheral nervous system

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Schwann cells (SCs) are the glial component of the peripheral nervous system, with essential roles during development and maintenance of axons, as well as during regenerative processes after nerve injury. SCs increase conduction velocities by myelinating axons, regulate synaptic activity at presynaptic nerve terminals and are a source of trophic factors to neurons. Thus, development and maintenance of peripheral nerves are crucially dependent on local signaling between SCs and axons. In addition to the classic mechanisms of intercellular signaling, the possibility of communication through secreted vesicles has been poorly explored to date. Interesting recent findings suggest the occurrence of lateral transfer mediated by vesicles from glial cells to axons that could have important roles in axonal growth and axonal regeneration. Here, we review the role of vesicular transfer from SCs to axons and propose the advantages of this means in supporting neuronal and axonal maintenance and regeneration after nerve damage.

Keywords: Schwann cell, axon, vesicular transfer, exosomes, microvesicles, axonal regeneration

INTRODUCTION

Originally, glial cells were considered as a sort of glue that filled the space between neurons, which is largely a passive role. In time, this view changed substantially. In the peripheral nervous system (PNS), Schwann cells (SCs) were recognized to regulate a wide variety of ongoing functions of axons (Mirsky and Jessen, 1999). It is well known that the myelin sheath, by increasing the operational resistance of the axolemma, greatly increases the velocity of the nerve impulse, which is a passive effect (Hartline and Colman, 2007).

A number of observations indicate that the cellular biology of axons is regulated by SCs. In the axolemma of unmyelinated fibers, sodium and potassium channels exist side by side (Garrido et al., 2003) but in axons surrounded by myelin, the axolemma under the sheath is poor in sodium and rich in potassium channels while the converse occurs at the nodal axolemma, where sodium channels accumulate (Salzer et al., 2008; Feinberg et al., 2010). This indicates that SCs regulate at a molecular scale the local organization of axons, thus regulating the axonal phenotype.

Nerve injury and its ensuing repair illustrate the mutual regulation of axons and SCs. Waller (1850) established that nerve section is followed by degeneration of the distal domain while SCs evolve to a dedifferentiated state. Nerve repair re-establishes the original condition (Jessen and Mirsky, 2008). After nerve injury, elongation of axons was shown to be prevented by SCs as long as they remained differentiated distal to injury (Tapia et al., 1995; Court and Alvarez, 2000), which strongly suggests that nerve repair proceeds in close interaction with the SC and not commanded by the cell body (Bray and Aguayo, 1974; Court and Alvarez, 2005). When SCs in a segment of an intact nerve are treated with a protease inhibitor, which has been shown to induce SC dedifferentiation (Alvarez et al., 1992, 2000; Tapia et al., 1995), the associated axon extends sprouts in that segment in spite of being surrounded by SCs, i.e., branches arise in an uninterrupted axon. This indicates that the axon has a growth program repressed by the differentiated SC (Court and Alvarez, 2005). Together, these phenomena illustrate that the SC locally affects the underlying axon, from its passive electrical properties, to organization of the axolemma, and even complex cellular programs embodied in the axoplasm.

We will consider now the first step of regulatory mechanisms between cells that operate on a local basis. Adhesion molecules are an important and well characterized mechanism that allows contact-mediated signaling between cells. Another mechanism involves extracellular free ligands that are produced by a cell and operate on a very short range, from its site of release to its receptor in the target cell. These two mechanisms share an important feature, namely, the machinery that produces the response belongs entirely to the target cell. A third regulatory mechanism has emerged in which a cell produces vesicles that are taken up by the target cell and the cargo is incorporated into the recipient cytoplasm (Simons and Raposo, 2009). This mechanism opens a new dimension to the intercellular interaction in that the recipient cytoplasm may contain an incomplete machinery that is completed by molecules of the donor cell upon their release from the vesicle. Our review focuses on the regulation of axons by SCs mediated by secreted vesicles and proposes the advantages of this means of communication in supporting neuronal and axonal maintenance and regeneration after nerve damage.

EVIDENCES FOR VESICULAR TRANSFER BETWEEN SCs AND AXONS

That proteins may enter the cytoplasm from the outside is an old notion. About fifty years ago, it was established that some proteins of the oocyte yolk of the mosquito *Aedes aegypti* were synthesized in the gut, moved to the ovary, and were taken up from the extracellular space via pinocytic vesicles to be stored essentially as a reservoir of amino acids for the embryo (Roth and Porter, 1964). In the nervous system, glia-to-axon transfer of protein was proposed about forty years ago. The giant axon of the squid was incubated with radiolabeled amino acids and labeled proteins were recovered from its axoplasm (Lasek et al., 1974). However, the notion of transcellular transfer emerged under the assumption that axons were unable to synthesize proteins, but since this assumption was wrong as axons do synthesize protein (Koenig and Giuditta, 1999; Alvarez et al., 2000; Donnelly et al., 2010; Gumy et al., 2010), the notion of glia-to-axon transfer of protein awaited further experimental support.

Around the 1980s, the groups of Stahl and Johnstone provided evidence to support that vesicles can mediate the release of proteins during reticulocytes maturation (Harding et al., 1983; Pan et al., 1985). These vesicles named exosomes were contained within multivesicular endosomes whose fusion with the plasma membrane was followed by exosome secretion (Johnstone et al., 1987; Simons and Raposo, 2009; Thery et al., 2009). In turn, vesicles originated after the evagination of the plasma membrane were named microvesicles (Cocucci et al., 2009; Thery et al., 2009). That was the beginning of a new era in cell communication, the release of membrane vesicles.

Based on these antecedents, transfer of macromolecules from SC to axons was reconsidered, this time mediated by vesicles. Thus, Buchheit and Tytell (1992) described transfer of fluorescently labeled vesicles from SCs to squid giant axons. They proposed that these vesicles carried the proteins previously thought to be transferred directly from the SC to the axon, such as heat shock protein (Hsp) 70 (Tytell et al., 1986) – a protein also carried in exosomes secreted by reticulocytes (Davis et al., 1986) – but they did neither confirmed these possibilities nor their functional significance. Nowadays, exosomes and microvesicles have been described in glial cells from the central nervous system (CNS, see **Table 1**), although in the PNS the evidence is scarce. Hsp70 is present in exosomes secreted from a SC cell line (Fevrier et al., 2004), SC primary cultures (Lopez-Verrilli M. A. and Court F. A., unpublished results) and in exosomes secreted by glial cells from the CNS, including astrocytes, oligodendrocytes, and microglia (Potolicchio et al., 2005; Krämer-Albers et al., 2007; Taylor et al., 2007). It remains to be investigated whether vesicular transfer of Hsp70 to axons confers neuroprotection to stress stimuli and neurodegenerative disorders.

Schwann cells are essential during regenerative processes after nerve injury, not only by secreting growth factors (Madduri and Gander, 2010; Quintes et al., 2010) but also by supplying components of the protein synthesis machinery to axons. Court et al. (2008, 2011) demonstrated *in vivo* the transfer of ribosomes from SCs to axons after axonal damage as well as during axonal regeneration. Electron microscope images showed ribosomes in the axoplasm but also within vesicles surrounded by two or multiple membranes. Interestingly, even multimembrane vesicles open to the axoplasm were still partially loaded with ribosomes and abundant free ribosomes seemingly discharged in the vicinity, suggesting that SC-derived vesicles were secreted and internalized in axons by endocytosis. Nevertheless, the molecular mechanism for ribosomal transfer after axonal damage and during axonal regeneration has not been disclosed yet.

Considering that SC exosomes diameter varies between 50 and 120 nm (Lopez-Verrilli M. A., and Court F. A., unpublished results), only a small amount of ribosomes could be transported within each exosome. On the other hand, microvesicles are larger vesicles (up to 1 μm; Cocucci et al., 2009) and might even transport polyribosomes. Since mRNAs can be stored in a dormant state in the distal axon until needed (Yoo et al., 2010), the transfer of mRNA-containing ribosomes from SC to axon could supply transcript for storage and translation in response to acute stimuli (e.g., nerve damage) or the transfer be triggered by the stimuli itself. In addition, vesicular transfer from SCs would accelerate the arrival of ribosomes to the axon, compared to ribosomes derived from the neuronal cell body (Twiss and Fainzilber, 2009).

In the dark side of vesicular transfer, SCs have been shown to secrete exosomes containing pathogenic prions upon cell infection *in vitro*, therefore prion secretion via SC-derived exosomes may spread these pathogenic proteins from the PNS to the CNS (Fevrier et al., 2004). Prions are misfolded proteins that act as infectious agents and cause neurodegenerative diseases (Weissmann et al., 2011). Furthermore, pathological cell–cell communication by endogenous vesicular vectors could be one of the mechanistic explanations for non-cell autonomous processes playing critical roles in neurodegenerative diseases (Garden and La Spada, 2012).

Summing up, SCs might provide by means of secreted vesicles, an efficient, specific, and highly localized support for axons. Vesicles interact specifically with the target cell (Rana and Zoller, 2011) supplying many copies and many kinds of macromolecules, which might allows SCs to locally regulate axonal functions without direct involvement of the neuronal cell body. Together, the evidence suggests that machineries of a given cytoplasm may be incomplete requiring the contribution of a neighboring cell to become operative (Court et al., 2008, 2011). From another point of view, the phenotype of a cell may require the contribution of an external genome to supply the missing messenger RNAs. SCs contain mRNAs coding for neurofilament proteins, which they barely translate (Roberson et al., 1992), and its transfer within vesicles could be instrumental for protein homeostasis in axons. In brief, the transfer of vesicles and their cargo of protein and RNAs open a novel mode for intercellular interaction, and a broad avenue of research.

POTENTIAL ROLES AND FUNCTIONS OF SECRETED VESICLES IN THE PNS

Vesicle secretion as a means to supply components to a target cell offers a number of advantages considering the SC and axon dynamics, e.g., during myelination and regeneration conditions.

In the myelinated nerve fiber (**Figure 1A**), vesicles could be released from microvilli domains to the node of Ranvier and transfer scaffolding proteins required for the proper node formation, such as actin, tubulin, cofilin, and ankyrin-G (Krämer-Albers et al., 2007; Valadi et al., 2007). Vesicles could be secreted along

Table 1 | Vesicles secreted by glial cells: content and effects upon target cells.

Cell of origin	Vesicle type	Recipient cell	Relevant vesicle content	Effect over recipient cell or secretion details	Reference
Schwann cells	Exosomes	ND	PLP, CNP, MBP, PrPc, PrPsc	Prions-infected SCs secrete infectious exosomes containing PrPsc	Fevrier et al. (2004)
	Unknown	Neuron (axonal compartment)	Ribosomes	Transfer of ribosomes stimulated by axonal damage and during regeneration	Court et al. (2008), Court et al. (2011)
Oligodendrocytes	Exosomes	ND	PLP, MOG, MBP, CNP	Oligodendrocytes secrete exosomes upon Ca^{+2} influx to the cytoplasm. Characterization of protein and lipid composition of oligodendrocyte derived exosomes	Krämer-Albers et al. (2007)
		ND	PLP	Exosome formation into multivesicular bodies is dependent of ceramide synthesis and independent of the ESCRT machinery.	Trajkovic et al. (2008)
		ND	PLP	Exosome secretion is regulated by Rab35 GTPase and its GAPs TBC1D10A–C	Hsu et al. (2010)
		ND	Flotillin-2, cholesterol	Exosomes containing flotillin-2 allow the discharge of cholesterol from oligodendroglial cells.	Strauss et al. (2010)
		Oligodendrocyte	PLP, MOG, MAG, CNP	Exosomes inhibit oligodendrocyte differentiation and myelin formation	Bakhti et al. (2011)
		Microglia	PLP, MOG, CNP	Oligodendrocyte derived exosomes are selectively internalized by microglial cells via macropinocytosis	Fitzner et al. (2011)
		Astrocytes	Hsp70, TRAIL	Oligodendroglial exosomes induce apoptosis in astrocytes	Lo Cicero et al. (2011)
	Exosomes and microvesicles	Cortical neurons	FasL, Nogo protein B	Vesicles induces apoptosis of cortical neurons	D'Agostino et al. (2006)
Astrocytes	Exosomes	ND	Hsp70	Astrocytes secrete exosomes containing Hsp70 upon heat shock stress	Taylor et al. (2007)
		ND	mtDNA	Exosomes from astrocytes and glioblastoma tumors secrete exosomes carrying mitocondrial DNA	Guescini et al. (2010)
		HUVECs	RNA and DNA, mutated and amplified oncogene sequences and transposable elements	Glioblastoma tumors secrete vesicles with functional RNAs and DNAs to potentially promote tumor progression	Balaj et al. (2011)
		ND	Synapsin	Extracellular synapsin stimulates neurite outgrowth	Wang et al. (2011)
	Microvesicles	ND	IL-1β	ATP acting on P_2X_7 increases microvesicle secretion from astrocytes, a mechanism dependent on sphingomyelinase activation	Bianco et al. (2009)
		ND	MMP2, MMP9 and TIMP2	MMP 2 and 9, and tissue inhibitors of MMP are released in microvesicles from astrocytes.	Sbai et al. (2010)
		ND	Ectonucleotidase NTPDase	Microvesicles containing NTPDase degrades extracellular ATP in an *in vitro* model of the blood brain barrier.	Ceruti et al. (2011)
	Exosomes and microvesicles	ND	β1-integrin, FGF-2, VEGF	Astrocytes secrete vesicles containing β1-integrin, FGF-2 and VEGF	Proia et al. (2008)

(Continued)

Table 1 | Continued

Cell of origin	Vesicle type	Recipient cell	Relevant vesicle content	Effect over recipient cell or secretion details	Reference
Microglial cells	Exosomes	ND	Aminopeptidase CD13	Characterization of exosome release and measurement of exosomal aminopeptidase CD13 activity.	Potolicchio et al. (2005)
		ND	IDE	Exosomes carrying IDE degrade extracellular β-amyloid peptide	Tamboli et al. (2010)
	Microvesicles	ND	IL-1β	ATP-derived astrocytes promotes microvesicle secretion by microglial cells	Bianco et al. (2005)
		Hippocampal neurons	ND	Microvesicles influence synaptic activity by increasing spontaneous and evoked excitatory transmission in neurons	Antonucci et al. (2012)
Glioblastoma cells	Exosomes	Endothelial and glioma cells	Angiogenic proteins, mRNAs, microRNAs and the tumor-specific EGFRvIII mRNA	Tumor vesicles mRNA content can be translated in endothelial and glioma cells, promoting angiogenesis, and tumor proliferation	Skog et al. (2008)
		Cancer cells lacking EGFRvIII	EGFRvIII	Exosomes carrying EGFRvIII transfer oncogenic activity to cancer cells lacking EGFRvIII and promote the expression of EGFRvIII-regulated genes	Al-Nedawi et al. (2008)
	Exosomes and microvesicles	ND	RNA	Vesicle-derived RNA analysis purified from serum of control and glioblastoma multiforme patients	Noerholm et al. (2012)

ND, not determined; CNP, 2′3′-cyclic-nucleotide-phosphodiesterase; EGFRvIII, truncated isoform of endothelial growth factor receptor; ESCRT, endosomal sorting complex required for transport; FasL, Fas ligand; FGF-2, fibroblast growth factor 2; Hsp70, heat shock protein 70; HUVECs, human umbilical vein endothelial cells; IDE, insulin-degrading enzyme; IL-1β, interleukin-1β; MBP, myelin basic protein; MMP, matrix metalloproteases; MOG, myelin oligodendrocytes glycoprotein; mtDNA, mitochondrial DNA; NTPDase, Nucleoside triphosphate diphosphohydrolase; PLP, proteolipid protein; PrP, prion protein; PrPsc; prion protein scrapie; TIMP2, metallopeptidase inhibitor 2; SCs, Schwann cells; TRAIL, tumor necrosis factor-related apoptosis-inducing ligand; VEGF, vascular endothelial growth factor.

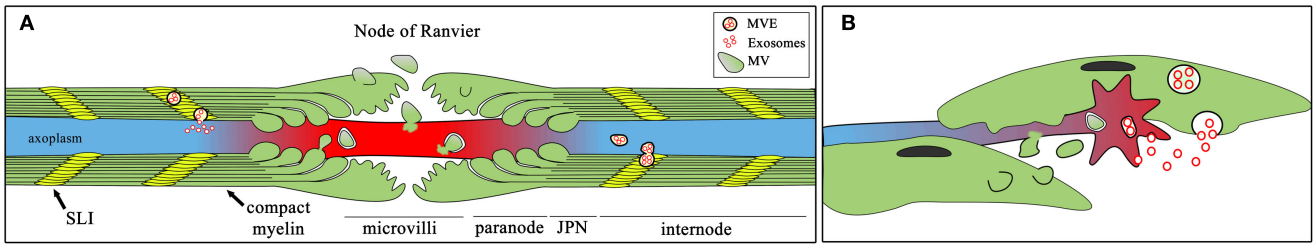

FIGURE 1 | Possible routes and conditions for SC to axons transfer of vesicles. Schematic representation of possible routes of exosomal (red circles) and microvesicle (larger green ovoids) transfer between SCs and axons in a myelinated fiber **(A)** and in an axonal growth cones during axonal regeneration **(B)**. Exosomes are contained within multivesicular endosomes (MVE) in the secreting cell, and then can move to the axon through cytoplasmic-rich region in SCs, including Schmidt-Lantermann incisures [SLI, yellow regions in **(A)**] or paranodal domains of myelinating fibers **(A)** or can be released close to the growth cone by dedifferentiated SC **(B)**. Microvesicles, in turn, are generated from the evagination of SC plasma membrane and they can fuse or be internalized by axons.

the Schmidt-Lantermann incisures cytoplasm channels across the myelin sheath or paranodal domains directly to the axoplasm, providing macromolecules in a temporally and spatially regulated fashion. Both exosomes and microvesicles deliver not only proteins but also microRNAs and mRNAs that can be translated into recipient cells (Ratajczak et al., 2006; Valadi et al., 2007). In fact, elongation factors needed for mRNA translation have been found in exosomes from oligodendrocytes and microglial cells (Potolicchio et al., 2005; Krämer-Albers et al., 2007).

Exosomes and microvesicles may actively participate in processes activated after nerve damage, when SCs dedifferentiate and begin to proliferate (**Figure 1B**). This scenario is quite complex since axons degenerate distal to the injury, while in the proximal stump, regeneration takes place (Coleman, 2005; Twiss and van Minnen, 2006). In these conditions, SCs could secrete vesicles containing mRNAs and microRNAs to negatively regulate myelination and stimulate proliferation, as observed for glial tumor cells (Skog et al., 2008). In addition, SCs-secreted vesicles could sustain protein synthesis in regenerating axons (Court et al., 2011), independently from axonal mRNA synthesized in the neuronal nuclei, which needs to be transported a long way before translation, or even provide guiding clues to regenerating axons, such as the axonal guidance protein Wnt, which has been detected in exosomes from motor neurons (Zou, 2004; Korkut et al., 2009).

If SC-derived vesicles are demonstrated to have functional roles in axonal regeneration, SC differentiation, or other processes crucial for neural tissue regeneration, these vesicles can be used for therapeutic purposes by using their endogenous potentials or by loading them with specific transcript or proteins by modifying glial cells, which can be easily manipulated *in vitro* (Schmitte et al., 2010; Zhang et al., 2010). It has been demonstrated that neuronal-targeted exosomes obtained from genetically modified dendritic cells *in vitro* can be electroporated with specific siRNA, and after intravenous injection, they specifically knock-down their target gene in brain neurons (Alvarez-Erviti et al., 2011). Vesicle-mediated drug delivery promises to overcome important challenges, such as delivery of drugs across impermeable biological barriers and using patient-derived cells to obtain tolerogenic vesicles.

CONCLUDING REMARKS

In this review we presented evidence for SC to axon communication via secreted vesicles and highlighted the functional role this process may have in the maintenance of peripheral axons and during regeneration. Increasing evidence is suggesting that axons have the ability to respond to a challenge autonomously from the cell body albeit under SCs regulation (Alvarez et al., 2000; Court and Alvarez, 2005). Since axons are of any length up to several meters, this anatomy clearly poses logistic problems. The evolutionary solution may be that SC packs the requisite components in a vesicle to convey its cargo to the axoplasm. We propose that vesicles secreted by SCs and transferred to axons are a major mechanism by which SCs locally support axonal maintenance and regeneration after nerve damage. We hope that the study of the processes will enrich our understanding of the cellular biology of the nervous system.

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